Developmental Biology 356 (2011) 108–109

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Concurrent Session 4: Cellular Mechanisms Driving Developmental Events

Program/Abstract # 18

Gbetagamma signaling is essential for migration of the posterior lateral line primordium in zebrafish

Hui Xu[a], Martine Behra[b], Shawn Burgess[c], Songhai Chen[a], Fang Lin[d]
[a]*University of Iowa, Iowa City, IA, USA*
[b]*University of Puerto Rico, San Juan, PR, USA*
[c]*NIH/NHGRI, Bethesda, MD, USA*
[d]*The University of Iowa Anatomy and Cell Biology, Iowa City, IA, USA*

In metazoans, collective cell migration is critical for development, tissue repair and cancer metastasis. The zebrafish posterior lateral line primordium (PLLP) has emerged as an effective vertebrate model for the study of such movement, as it makes genetic manipulation and in vivo imaging possible. Previous studies showed that the PLLP migrates along a track of cells expressing the chemokine Sdf1a, and that proper migration is governed by asymmetrical expression of its G protein-coupled receptors, Cxcr4b and Cxcr7, within cells of the PLLP. However, how G proteins and their downstream effectors contribute to this migration remains poorly understood. Here we report that signaling by the G protein bg subunits is essential for proper PLLP migration. We show that Gβ1 isoforms (Gβ1a and Gβ1b) are highly expressed in the migrating primordium, and that morpholino-mediated knockdown of Gβ1 expression severely impairs PLLP migration. Specifically, Gβ1-depleted PLLP migrates only a minimal distance, if at all, and then rounds up; this phenotype resembles that observed in embryos with Cxcr4b deficiency. Confocal time-lapse imaging reveals that whereas the leading cells of the WT migrating PLLP have extensive actin-rich fidipodia and pseudopodia, Gβ1-depleted counterparts form only "bleb"-like protrusions. Intriguingly, transplantation of WT cells into the leading region of the Gβ1-depleted PLLP fully restores migration, whereas transplantation into the trailing region does not. Thus, we propose that Gβγ signaling is required in cells of the leading region for the proper response to chemokine signaling. Currently, we are investigating the molecular mechanisms by which Gβγ signaling regulates PLLP migration.

doi:10.1016/j.ydbio.2011.05.029

Program/Abstract # 19

Roles of localized mRNAs in lipid droplet function during cortical rotation in *Xenopus*

Douglas W. Houston[a], John Olthoff[b], Olson David[b]
[a]*The University of Iowa Department of Biology, Iowa City, IA, USA*
[b]*The University of Iowa, Iowa City, IA, USA*

Dorsal axis formation requires the localized activation of Wnt/beta-catenin signaling on the future dorsal side of the blastula. This asymmetry in Wnt signaling is established by rotation of the egg cortex about the deep cytoplasm during the first cell cycle. Cortical rotation is a microtubule-based event, and is thought to result in the localization of dorsal determinants, including wnt11 mRNA and intracellular activators of Wnt/beta-catenin signaling. Despite the importance of cortical rotation, the mechanisms controlling microtubule assembly and alignment are unknown and it is unclear to what extent these events are linked to the initiation of Wnt signaling. Using maternal mRNA depletion, we have recently shown that the ubiquitin ligase activity of Trim36, a RING finger protein encoded by a vegetally-localized mRNA, is essential for cortical rotation and dorsal Wnt target gene expression. Interestingly, these phenotypes are similar to those reported for depletion of maternal Adrp/Fatvg, a lipid droplet protein encoded by another vegetally-localized mRNA. We present evidence that Trim36 is associated with subsets of vegetal organelles and that Trim36 and Adrp functionally interact to control microtubule assembly during cortical rotation. Additionally, we present work to identify the role of Trim36 in lipid droplet and organelle function and to identify targets of Trim36 ubiquitylation.

doi:10.1016/j.ydbio.2011.05.030

Program/Abstract # 20

Src64 regulates myosin regulatory light chain during basal closure of the *Drosophila* cellular blastoderm

Jeffrey H. Thomas[a], Rafael Rosales[b], Ashish Chougule[b]
[a]*Texas Tech Univ Health Sci Ctr Cell Biology & Biochem, Lubbock, TX, USA*
[b]*Texas Tech Health Sciences Center, Lubbock, TX, USA*

After fertilization, the *Drosophila* embryo does not undergo cleavage, but instead undergoes syncytial nuclear division. After the nuclear division cycles are completed, approximately 6000 nuclei at the periphery of the embryo are surrounded by plasma membrane, forming the cellular blastoderm. The bases of the newly-formed cells are partially closed by the constriction of actomyosin rings to form stalks connecting the cells to the underlying yolk sac. Basal closure requires src64, a Src nonreceptor tyrosine kinase. The actomyosin rings appear morphologically normal in src64 mutant embryos, but do not constrict. We find that phosphorylation of the regulatory subunit of nonmuscle myosin II, myosin regulatory light chain (MRLC), is reduced in src64 mutants. MRLC (spaghetti squash) mutant embryos have highly disorganized actomyosin cytoskeletons during cellularization: basal actomyosin rings do not properly form, and we observe no evidence of actomyosin contraction. Expression of constitutively-active MRLC in src64 mutant embryos rescues the actomyosin ring constriction defects, suggesting that Src64 regulates myosin activity during cellular blastoderm formation. Another

protein, Rho kinase (Rok), has been implicated in the regulation of MRLC phosphorylation and actomyosin contraction. However, we observe that, despite the disorganization of the actomyosin cytoskeleton in Rok mutant embryos, actomyosin ring constriction occurs in embryos that undergo cellularization. Thus, our observations suggest that Src64 regulates myosin activity either independently of Rok, or through the activities of both Rok and other, functionally redundant, MRLC regulators.

doi:10.1016/j.ydbio.2011.05.031

Program/Abstract # 21

Signaling and mechanics: Extracellular ATP regulates global gastrulation movements by controlling epithelial contractility

Lance Davidson, Mike von Dassow, Sagar Joshi
University of Pittsburgh, Pittsburgh, PA, USA

Cell contractility is a major tissue shaping process during epithelial morphogenesis. Embryonic cells are able to dynamically remodel their cytoskeleton and induce cell shape change resulting in changes in tissue architecture. Perfusing cell lysate over the epithelial surface of developing embryos induces localized contraction. Chronic exposure to cell lysate produces severe developmental defects, arresting blastopore closure and driving embryos to exogastrulate. We have identified extracellular ATP as the ligand responsible for both acute and chronic responses to cell lysate. We present here the physical mechanics of inducible epithelial contractility and the pathway that controls and modulates the contractile response. This pathway offers an example of a robust mechanotransduction pathway for regulating embryonic epithelial mechanics that is analogous to physiological pathways that maintain epithelial contractility.

doi:10.1016/j.ydbio.2011.05.032

Program/Abstract # 22

Determining the role of the centrosome in establishing epithelial cell polarity

Jessica Feldman, James Priess
Fred Hutchinson Cancer Research Center/HHMI Basic Sciences, Seattle, WA, USA

The centrosome is the major microtubule organizing center (MTOC) of the cell. However, MTOC function is often reassigned from an apically positioned centrosome to the neighboring plasma membrane in epithelial cells. We are interested in understanding how centrosomes become apically positioned and how, or if, the apical positioning of the centrosome is involved in the reassignment of MTOC function. In the development of the *C. elegans* intestinal epithelium, centrosomes shift from an anterior or posterior position following the final round of division to an orthogonal position at the future apical surface. This repositioning occurs at a developmental stage when cells are just beginning to polarize, and lack hallmarks such as apical junctions. We find that, prior to centrosome repositioning, polarity proteins such as PAR-6 and PAR-3 accumulate in foci at anterior or posterior membranes, and that these foci traffic with centrosomes to the future apical surface. While PAR-6 is not required for centrosome repositioning, PAR-3 appears to be required for this process; maternal and zygotic depletion of PAR-3 results in centrosome positioning defects. Microtubules are also required for centrosome repositioning as treatment of embryos with microtubule depolymerizing drugs can inhibit the initiation and progression of centrosomes to the future apical surface. During normal repositioning, microtubule-nucleating proteins normally found at the centrosome such as γ-tubulin and grip-1 become deposited along the apical surface of intestinal cells, consistent with this surface becoming an MTOC. Remarkably, live cell imaging suggests that the apical γ-tubulin originates from the centrosome. In embryos lacking PAR-3, where centrosomes fail to reposition apically, we see an aberrant localization of γ-tubulin and grip-1. Together, these data suggest that one function of centrosome repositioning might be to hand-off nucleators of microtubule assembly to the apical surface prior to polarization. We are now trying to eliminate centrosomes in intestinal cells to determine if they are essential for MTOC reassignment.

doi:10.1016/j.ydbio.2011.05.033